

We Claim:

- Siba A* > 1. A method of biological assay comprising:
5 a) providing an enzyme substrate comprising two or more fluorescence dye groups bound to a flexible peptide comprising one or more enzymatically cleavable bonds, the dye groups being of proximity sufficiently close to allow free energy attractions to draw the dye groups together so as to essentially self-quench fluorescence of the dye groups, wherein self quenching of fluorescence of the dye groups is effected by dye stacking, and
10 b) enzymatically cleaving one or more of said cleavable bonds of the peptide to release the fluorescence dye groups from dye stacking, thereby producing an increase in fluorescence intensity.
- 15 2. ~~The method according to claim 1 wherein said dye groups are identical.~~
3. The method according to claim 1 wherein said dye groups comprise a fluorescence donor and acceptor.
- 20 4. The method according to claim 1 wherein said dye groups are separated from each other by a distance of less than 100 Å.
5. The method according to claim 1 wherein said released fluorescence dye groups emit radiation in the visible range.
- 25 6. The method according to claim 1 wherein said fluorescence dye groups have a planar configuration.
7. The method according to claim 1 wherein said dye groups are selected from
30 the group consisting of fluorescein, rhodamine, and cyanine dye groups.

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8. The method according to claim 1 wherein said dye groups are selected from the group consisting of fluorescein, tetramethylrhodamine, X-Rhodamine, Rhodamine B, and TEXAS RED.
- 5 9. The method according to claim 1 wherein said flexible peptide comprises from 2 to about 10 amino acids, wherein said dye groups bound to said peptide form a dye dimer or stack and wherein said peptide has at least one enzyme-specific cleavable bond.
- 10 10. The method according to claim 1 wherein said enzyme involved in said enzymatic cleaving is selected from the group consisting of aspartic, metallo-, thiol, serine, retroviral, and trypsin proteases.
- 15 11. ~~The method according to claim 1 wherein said fluorescence intensity is increased at least 10-fold compared to the intensity increase in conventional assay kits using a protease substrate.~~
- 20 12. A protease substrate comprising a flexible peptide and including two fluorescence dye groups, the dye groups being of sufficiently close proximity to allow free energy attractions to draw the dye groups together so as to essentially self-quench fluorescence of the dye groups by intramolecular dimerization or stacking.
- 25 13. The protease substrate according to claim 12 wherein said dye groups of an intramolecular dimer formed by said intramolecular dimerization are separated by a distance of less than 100Å.
- 30 14. ~~The protease substrate according to claim 12 wherein said dye groups are identical.~~
15. The protease substrate according to claim 12 wherein said dye groups comprise a fluorescence donor and acceptor.

16. The protease substrate according to claim 12 wherein said dye groups have a planar configuration.

5 17. The protease substrate according to claim 12 wherein said dye groups are selected from the groups consisting of fluorescein, rhodamine, and cyanine dye groups.

10 18. The protease substrate according to claim 12 wherein said dye groups are selected from the groups consisting of fluorescein, tetramethylrhodamine, X-Rhodamine, Rhodamine B, and TEXAS RED.

15 19. The protease substrate according to claim 12 wherein said peptide comprises from 2 to about 10 amino acids, wherein said dye groups bound to said peptide form a stack, and wherein said peptide has at least one enzyme-specific cleavable bond.

20. The protease substrate according to claim 12 having the formula of SEQ ID NO. 2:

20 TMR-Val-Pro-Arg-Gly-Lys-TMR.

Juf A3 > 21. An assay method of detecting a microorganism, which microorganism produces a characteristic enzyme, comprising:

25 a) providing an enzyme substrate specific for said characteristic enzyme comprising two or more fluorescence dye groups bound to a flexible peptide comprising one or more bonds cleavable by said characteristic enzyme, the dye groups being of proximity sufficiently close to allow free energy attractions to draw the dye groups together so as to essentially self-quench fluorescence of the dye groups, wherein self quenching of fluorescence of the dye groups is effected by dye dimerization or stacking, and

- b) cleaving one or more of said cleavable bonds of the peptide by said characteristic enzyme to release the fluorescence dye groups from dye dimerization or stacking, thereby producing an increase in fluorescence intensity.

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